

ANTI-SELECTIN ANTIBODIES FOR PREVENTION OF MULTIPLE ORGAN
FAILURE AFTER POLYTRAUMA AND FOR PREVENTION OF ACUTE
ORGAN DAMAGE AFTER EXTRACORPOREAL BLOOD CIRCULATION

FIELD OF THE INVENTION

5 This invention relates to the use of anti-selectin
antibodies for the prevention of multiple organ failure
associated with polytrauma and for the prevention of acute
organ damage associated with extracorporeal blood
circulation. Especially preferred are antibodies to E-
10 selectin, L-selectin, and/or P-selectin.

BACKGROUND OF THE INVENTION

15 A polytrauma is understood as an injury of a number of
tissues (bones or soft tissues). In a polytraumatic event,
mediator systems (e.g. cytokines, arachidonic acid
products, oxygen radicals, proteases) as well as leukocytes
and other cells are activated. This can lead to secondary
organ damage (e.g. destruction of tissue structures by
liberated proteases). This secondary organ damage can
occur in the whole body independently of the site of the
20 primary trauma.

25 A polytrauma may also be associated with hemorrhagic
shock. Hemorrhagic shock is understood as a shock which is
characterized by a rapid and substantial loss of blood
toward the inside or outside. At present, hemorrhagic
shock can be treated successfully by intensive medical
therapy, especially by volume substitution and blood
transfusion. The combination of hemorrhagic shock and

trauma is referred to as hemorrhagic-traumatic shock. In contrast to pure hemorrhagic shock, there is, at present, no specific therapy for traumatic or hemorrhagic-traumatic shock and no prophylaxis at all for later organ failure following polytrauma.

Multiple organ failure (MOF) is a severe problem which often occurs after polytraumas. The more organs affected, the higher the mortality. Organs and systems which can fail include the heart, lung, kidney, liver, stomach, intestinal system and central nervous system. Although in recent years it has been possible to reduce the very high mortality of trauma patients to about 20 % by improvements in rescue service and emergency medicine, so far there is no specific therapy for organ failure.

Marzi et al., J. Trauma 35: 110-119 (1993) discloses that superoxide dimutase can be given 24 hours after trauma. However, the results are not unequivocal and merely show a trend towards a partial improvement. A substantial reduction in mortality and MOF was, however, not observed. Mileski, W.J. et al., Surgery 108: 206-212 (1990) discloses that the binding of neutrophils or their aggregation contributes substantially to the development of hemorrhagic shock after organ damage. In this case, experimental animals were given anti-CD18 antibodies immediately after a 90 minute phase of hemorrhagic shock. Therapeutic methods for treatment of multiple organ failure after polytrauma is not described by Mileski. Vedder N.B.

et al., Surgery 106: 509-516 (1989) also propose the use of anti-CD18 antibodies to treat hemorrhagic shock.

Selectins, such as L, E, and P-selectin, have been found to be associated with tissue damage during the course of ischemia and reperfusion. Neutrophils play an important role in this connection. It is assumed that selectin is required for the recruitment of neutrophils. Apparently L-selectin is necessary for the complete development of damage in skeletal muscle as well as in the lung (Seekamp A. et al., Am. J. Pathol. 11: 592-598 (1994). Mulligan, M.S. et al., J. Immunol. 151: 832-840 (1994) describe a similar phenomenon.

The production of humanized anti-L-selectin antibodies is described in WO 94/12215, incorporated herein by reference. The use of such antibodies in the treatment of inflammatory diseases and in particular of myocardial infarction is proposed. A dose of 1 - 50 mg is proposed to prevent acute lung failure. However, the reference does not describe a method for preventing MOF after polytrauma.

Thus, there is a need for effective treatment of preventing and/or treating multiple organ failure after polytrauma.

Acute organ damage can also be caused during cardiovascular surgery, such as an aorto-coronary vein bypass operation or a cardiac valve operation, where the blood of the patient circulates extracorporeally through a

heart-lung machine. The extent of the damage depends upon the period during which the machine is in operation. This can lead, e.g., to failure of the lungs, which can necessitate artificial respiration of the patient well after the operation (Birnbaum, D. et al., Z. Kardiol. 79, Suppl. 4: 87 - 93 (1990)). Other organs, such as the heart, kidneys, liver or systems such as the blood and coagulation system may also be damaged and fail.

It is known from Mulligan, M.S. et al., J. Immunol. 151: 832-840 (1994) that molecules which promote adhesion such as L, E, and P-selectins are involved in acute inflammatory processes. These molecules mediate the adhesive interaction of leukocytes with endothelial cells. In this connection L-selectin seems to play an important role in the initial phase (rolling) of acute intrapulmonary inflammatory reactions. Mulligan states further that anti-L-selectin antibodies are suitable for shortening the duration of the lung damage that can be triggered by L-selectin.

However, up to now no preventive therapy is known which can be used to prevent acute organ damage that is caused by extracorporeal circulation of the blood. Thus, there is a need for effective treatment of preventing acute organ damage caused by extracorporeal circulation of the blood.

OBJECTS OF THE INVENTION

An object of the invention is to provide a method and a therapeutic composition which can be used to effectively prevent multiple organ failure after polytrauma in humans and to considerably reduce the mortality rate of polytrauma patients. The invention concerns the use of anti-selectin antibodies therapeutically and for the production of pharmaceutical compositions useful in the prevention of multiple organ failure and death after polytrauma.

An object of the invention is also to provide a method and use of a therapeutic composition which contains anti-selectin antibodies for the prevention of acute organ damage after extracorporeal circulation. Such organ damage can be largely avoided with this method and this procedure. A particular advantage of the method is the extracorporeal application which leads to an effective decrease in organ complications.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows the lung wet weight of experimental animals with respect to the observation time.

Fig. 2 shows the cardiovascular parameter CO (cardiac output) with respect to time for the various experimental animals.

Fig. 3 shows the cardiovascular parameter MAP (mean arterial blood pressure) with respect to time for the experimental animals.

Fig. 4 shows the BE value (arterial base excess) with respect to time for the experimental animals.

Fig. 5 shows the number of white blood cells with respect to time for the various experimental animals.

5 DETAILED DESCRIPTION OF THE INVENTION

10 The invention concerns the use of at least one anti-selectin antibody for the production of a pharmaceutical composition to prevent acute organ damage after extracorporeal circulation of a patient's blood through a heart-lung machine, wherein 1 to 30 minutes before ending the extracorporeal circulation the anti-selectin antibody is added extracorporeally into the tube system of the heart-lung machine at a dose of 1.0 - 10 mg/kg of body weight of the patient and preferably 2 - 4 mg/kg.

15 Surprisingly, acute organ damage after extracorporeal circulation of a patient's blood can be prevented to a large extent by this preventive extracorporeal administration. In a preferred embodiment a total of 1 - 20 3 further doses of 1 - 4 mg/kg anti-selectin antibody, such as an anti-L-selectin antibody, are administered to the patient within 1 - 3 days. Polyclonal or monoclonal, murine, human, chimeric or humanized antibodies/immunoglobulins and their binding fragments can 25 be used as the anti-selectin antibodies. In one aspect of the invention, the therapeutic compositions are not administered into the body of the patient, but

extracorporeally, i.e., directly into the tube system of a heart-lung machine.

"Anti-selectin antibodies", as used herein, refers to any antibody which binds to a selectin. Especially preferred are antibodies which bind specifically to one of L-selectin, E-selectin, or P-selectin, as well as combinations of these. Also preferred are antibodies which react with more than one selectin, such as antibodies which react with both L- and E-selectin. L-selectin is a known glycoprotein that is constitutively expressed by all leukocytes. Both L-selectin and its murine homologues, GP90 and Mell4, are involved in the normal recirculation of lymphocytes - each mediates the interaction between circulating lymphocytes and vascular ligands (often referred to as "addressins") on the high endothelial venules (HEVs) of lymphoid organs (L.A. Lasky, et al., Cell 69: 927-938 (1992); E.L. Berg, et al., J. Cell. Biol. 114: 343-349 (1991)). In addition to its role as a lymphocyte homing receptor, L-selectin is also involved in the adhesion of circulating leukocytes to non-lymphoid tissues, such as endothelium, during inflammation. L-selectin is shed from the leukocyte surface following leukocyte activation (T.K. Kishimoto, et al., Science 245: 1238-1241 (1989)), and this may be an important process in retaining activated leukocytes at sites of inflammation. L-selectin has an amino-terminal carbohydrate-recognition domain (CRD) that has considerable homology with C-type lectins (K. Trickhamer, J. Biol. Chem. 263 9557-9560 (1988)), followed by a single epidermal-growth-factor-like domain,

complement-regulatory domains, a single transmembrane polypeptide and a carboxy-terminal cytoplasmatic domain. L-selectin interacts with its cognate ligand through the amino-terminal CRD in a calcium dependent manner.

5 In accordance with the invention, anti-selectin antibodies are preferred which modulate, and more preferably inhibit, the interaction between the CRD domain and the corresponding carbohydrate receptors on the surface of cells. Such carbohydrate receptors are described by R.B. Parekh, Tibtech 12: 339-345 (1994), incorporated by reference. These carbohydrate receptors may be phosphorylated or sulfated sugars.

10 In a further embodiment of the invention, anti-P- and/or anti-E-selectin antibodies are used instead of, or in addition to, anti-L-selectin antibodies. Such antibodies can be produced using P- or E-selectin (described in R.B. Parekh and T.F. Tedder, FASEB Journal 9: 866-873 (1995), incorporated by reference). In an especially preferred embodiment of the invention, anti-P- and/or anti-E-selectin antibodies are used which show considerable cross-reactivity with L-selectin antibodies, especially cross-reactivity with antibody HuDreg-55 or HuDreg-200.

20 As used herein, the term "humanized immunoglobulin" refers to an immunoglobulin comprising a human framework, at least one complementarity determining region (CDR) from a non-human antibody, and in which any constant region

present is substantially identical to a human immunoglobulin constant region, i.e., at least about 85-90%, preferably at least 95% identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDRs, are substantially identical to corresponding parts of one or more native human immunoglobulin sequences. For example, a humanized immunoglobulin would not encompass a chimeric mouse variable region/human constant region antibody. See, e.g., European Patent Application EP A 451216, incorporated by reference

The invention also concerns the use of such anti-selectin antibodies to reduce MOF and mortality after polytrauma. It has surprisingly turned out that it is possible to prevent multiple organ failure when anti-selectin antibodies, especially anti-L-selectin antibodies, are administered very soon after the polytrauma. This is also surprising because there are no acute symptoms at this early stage and there would therefore have been no reason to administer such a dose as a preventive measure.

It also has surprisingly turned out that anti-selectin antibodies in doses of 1.0 - 10 mg/kg, preferably of 2 - 4 mg/kg, administered one to five times, preferably once or twice after the polytraumatic event can advantageously be used, where the first application is given as early as possible, preferably 0.5 - 8 hours, and especially preferably, 0.5 - 4 hours after the polytraumatic event. The intervals between the individual applications are

between about 6 and about 72 hours, preferably between 6 and 36 hours.

In a preferred embodiment the dose and time of the second and subsequent preventive applications is selected depending on the concentration of the anti-selectin antibodies in the blood and preferably in plasma or serum, which is an early determinable parameter. In this connection it is preferable that the plasma concentration of the anti-selectin antibody is maintained at 10 - 100 $\mu\text{g/ml}$ over a time period of 7 - 10 days after the polytraumatic event. This concentration is equivalent to about a 10 - 100 fold excess over the concentration of soluble selectin in plasma. The dose and time for the second and subsequent applications are determined by determining the concentration of the anti-selectin antibody in blood, serum or plasma at intervals of 6 - 24 hours and immediately administering a dose which essentially corresponds to the dose of the first application when the plasma concentration falls below 10 $\mu\text{g/ml}$ antibody. When the antibody concentration is between 10 and 50 $\mu\text{g/ml}$, the antibody is administered at about half the concentration of the first application, and at an antibody concentration between 50 and 100 $\mu\text{g/ml}$, no further antibody is administered. In this case only the antibody concentration is monitored further.

The anti-selectin antibody concentration in blood, serum or plasma is determined by the usual methods, preferably by an immunological method of determination.

Such methods are known to a person skilled in the art. For example the determination can be carried out by means of an ELISA test in which a labelled selectin specific antibody, preferably the antibody which is also used therapeutically, competes for a specific selectin. In a subsequent step the amount of labelled antibody which has bound to the antigen is then determined and the concentration of the anti-selectin antibody in the sample is determined from this.

The therapeutic compositions of the invention are usually administered parenterally such as intravenously, intraarterially, intraperitoneally, subcutaneously or intramuscularly. Intravenous (i.v.) administration is preferred. The active components of the composition can be used in a liquid or solid form, preferably in a lyophilized form and be used together with a suitable diluent or carrier such as water or aqueous solutions of sodium chloride, dextrose, buffers and so forth. Other suitable pharmaceutical auxiliary substances can also be added.

Antibodies to selectin are known from the state of the art and are described for example in EP-A 0 386 906, WO 93/00111 and WO 94/12215 and by Kishimoto, T.K. et al., in Blood 78: 805-811 (1991) and Proc. Natl. Acad. Sci. USA 87: 2241-2248 (1990), all of which are incorporated by reference. L-selectin is also denoted LECAM-1, Mel 14 or Lam-1 in the literature. The cloning and sequence of Lam-1 have been described in WO 93/02698. Antibodies which bind specifically to selectin are suitable. Humanized

antibodies, especially HuDreg 200 which is described in WO 94/12215 and is expressly incorporated herein by reference, are suitable. Other antibodies which bind to selectin, such as HuDreg 55, (sequence: SEQ ID NO: 1 - 4), are also particularly preferred.

"Antibody" as used herein is understood as a protein that is composed of one or several polypeptide chains which are essentially encoded by antibody genes. The antibody genes code for the antigen-specific variable regions and may also code for the genes for the constant regions. Antibodies within the sense of the invention are also understood as various derivatives and fragments of antibodies such as Fv, Fab and F(ab)₂ and individual antibody chains (Houston et al., PNAS USA 85 5879-5883 (1988), Bird et al., Science 242: 423-426 (1988), Hood et al., Immunology, Benjamin N.Y., 2nd edition (1984), Hunkapiller and Hood, Nature 323 15-16 (1986)). Monoclonal antibodies and fragments thereof are preferably used and particularly preferably chimeric or humanized antibodies preferably of the IgG1 or IgG4 subtype.

The antibodies preferably contain at least two light polypeptide chains and two heavy polypeptide chains. Each of these chains contains a variable region (usually the N-terminal part of the polypeptide chain) which in turn contains a domain which binds the antigen. Heavy and light chains additionally contain a constant region of the polypeptide (usually the C-terminal part) which mediates the binding of the antibody to leukocytes (neutrophils,

lymphocytes etc.). Usually the light and heavy chains are complete antibody chains which are composed of the variable region and the complete constant region. In this connection, the variable regions and the constant regions can be derived from different antibodies, for example different isotypes. For example a polypeptide which contains the variable region of a heavy chain of an anti-selectin antibody of the γ -1 isotype may be linked to the constant region of the heavy chain of an antibody from another class (or subclass).

Anti-selectin antibodies are also suitable in which one or several amino acids are substituted. In this case, amino acids are preferably substituted by other amino acids with similar characteristic features (e.g. the acidic amino acid Asp by the acidic amino acid Glu). The structural characteristics of the original sequence are not changed by such substitutions. Examples of such polypeptide structures are described in Proteins, Structures and Molecular Principles, Creighton (editor), W.H. Freeman and Company, New York (1984); Introduction to Protein Structure, C. Brandon and J. Tooze, Garland Publishing, New York (1981); Thornton et al., Nature 354 105 (1991). In general, antibodies which are suitable as anti-selectin antibodies are those which bind to one or more of L-selectin, E-selectin, and P-selectin and/or inhibit the rolling of leukocytes (e.g. neutrophils).

In addition to the humanized immunoglobulins specifically described herein, other "substantially

homologous" modified immunoglobulins can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. Human antibodies, including, for example, the Eu or GAL antibodies, as well as other human antibodies known in the art, can be used as a source of framework sequence. These framework sequences should exhibit a high degree of sequence identity with the mouse Dreg 55 or mouse Dreg 200 variable region domains from which the CDRs were derived. The heavy and light chain variable framework regions can be derived from the same or different human antibody sequences. Indeed, the heavy and light chain framework regions can each be derived from more than one human antibody. The human antibody sequences can be the sequences of naturally occurring human antibodies or can be consensus sequences of several human antibodies. See Carter et al., WO 92/22653 (1992), incorporated by reference.

"Antibodies which are capable of binding in an equivalent manner" are understood as those antibodies which bind to the same or an overlapping epitope of a selectin. Epitope overlap can be determined by methods known in the art, for example with the aid of a competitive test system.

A competitive binding assay may be carried out for this and the extent to which the antibody competes with, e.g., HuDreg 55 for binding to an immobilized L-selectin antigen is determined. For this, L-selectin immobilized in a suitable manner (preferably L-selectin on leukocytes) is incubated with HuDreg 55 in a labelled form and an excess

of the antibody to be tested. The extent of the binding of the antibody to be tested to L-selectin is determined in comparison to HuDreg 55 by determining the bound label of the anti-leucocyte-bound label. If the labelled HuDreg 55 is displaced by at least 50 % by the antibody to be tested an epitope overlap is present. Antibodies that bind in an equivalent manner as HuDreg 55 are preferred for use in the invention.

"Antibodies which are capable of binding in an equivalent manner" can also be identified by screening for the capacity to block neutrophil-endothelial cell interaction. A simple visual assay for detecting such interaction has been described by Kishimoto et al. (*Blood*, 78:805 (1991)). Briefly, monolayers of human umbilical vein cells are stimulated with interleukin-1. Neutrophils, with or without pretreatment with the antibody under test, are added to the monolayer under defined conditions, and the number of adhering neutrophils is determined microscopically. In one method, the neutrophils are obtained from human leukocyte adhesion deficient patients. See Anderson et al., *Ann. Rev. Med.* 38:175 (1987). The neutrophils from such patients lack integrin receptors, whose binding to neutrophils might obscure the effects of blocking L-selectin binding.

The antibodies can be used as complete monoclonal antibodies, fragments thereof (Fv, (Fv)₂, Fab', F(ab')₂), chimeric, humanized or human antibodies. Short antibody fragments which only contain the CDR regions or parts

thereof which bind specifically to L-selectin can also be used.

The production of antibodies and in particular of monoclonal antibodies and fragments thereof is familiar to a person skilled in the art and described for example in E. Harlow and D. Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press (1988), Bessler et al., *Immunobiol.* 170: 239-244 (1985), Jung et al., "Angewandte Chemie" 97: 883 (1985), Cianfiglia et al., *Hybridoma* Vol. 2: 451-457 (1993).

Anti-selectin antibodies that can be used according to the invention can also be produced by recombinant means. Such processes are described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd edition (1989), Cold Spring Harbor, New York, Berger and Kimmel, *Methods in Enzymology*, Vol. 152, *Guide to Molecular Cloning Techniques* (1987), Academic Press Inc., San Diego CA, which are incorporated by reference. Such recombinant antibodies can be produced either in eukaryotic or prokaryotic cells by processes known to the art. Mammalian cells, especially lymphocytic cell lines, are preferably used as host cells. Chimeric, humanized or human antibodies are preferably produced by recombinant methodology. Regions can be selected for the non-antigen binding regions of the antibodies which are for example described in E.A. Kabat et al., *Sequences of Proteins of Immunological Interest* (1987), National Institute of Health, Bethesda MD. The production of recombinant anti-L-selectin antibodies of

humanized and human antibodies is described in WO 94/12215, which is hereby incorporated by reference. A particularly preferred, humanized anti-L-selectin antibody is HuDreg 55, which may be constructed in the same manner as HuDreg 200 described therein, and comprises two light chains having the sequence SEQ ID NO: 2 and two heavy chains having the sequence SEQ ID NO: 4.

Preferred humanized immunoglobulins are those which bind to selectin with a binding affinity of at least 1×10^7 M^{-1} in standard binding conditions (e.g., phosphate-buffered saline with 2 percent fetal bovine serum at 25°C). Examples of such humanized immunoglobulins are HuDreg 55 and HuDreg 200. More preferred are humanized antibodies, which preferably bind, in standard binding conditions, to human selectin with an affinity of at least 1×10^8 M^{-1} , and more preferably, with an affinity of at least 1×10^9 M^{-1} , and advantageously with an affinity of at least 1×10^{10} M^{-1} or more. Usually, the binding affinity of a humanized immunoglobulin is within a factor of 3-10 of the mouse immunoglobulin from which it was derived. For example, the affinity of the mouse Dreg 200 antibody is about 10^8 M^{-1} and that of mouse Dreg 55 is about 10^9 M^{-1} .

The following examples, sequence protocols, publications and figures further elucidate the invention. The described processes are to be understood as examples of illustration, not of limitation, which also after modifications, describe the subject matter of the invention.

EXAMPLE 1 - Use Of Anti-L-Selectin Antibody To
Reduce Post-Trauma Organ Failure

5 The protective action of a humanized antibody against
L-selectin (anti-L-selectin) in reducing post-traumatic
organ failure such as that which typically occurs after
injury in patients with severe polytrauma is demonstrated.
Humanized anti-L-selectin antibody (HuDreg 55) is used as
the antibody. It also reacts with baboon L-selectin. This
10 mouse form of this antibody is described by Kishimoto PNAS,
USA 87 (1990) 2244-2248. The humanized sequence is shown
in SEQ ID NO: 1 - 4.

15 The HuDreg 55 and HuDreg 200 antibodies react with
L-selectin on human leukocytes; however, only HuDreg 55
reacts with L-selectin of baboon leukocytes. Therefore
HuDreg 55 was used. Since HuDreg 55 and HuDreg 200 bind in
the same concentration range to human leukocytes (e.g. in
FACS analysis), the effects of HuDreg 55 on baboons is
presumptively equivalent to the effect of HuDreg 200.

20 As a model, severe tissue damage with associated
hypovolemia (= loss of liquid and blood towards the inside

and/or outside) was induced in baboons. The pure blood loss with subsequent shock (hemorrhagic shock) is less relevant for the lung damage (Pretorius et al., J. Trauma 1987; 27: 1344 - 1353; Schlag et al., page 384-402, in Schlag, Redl: Pathophysiology of Shock, Sepsis, and Organ Failure, Springer Verlag, Berlin, 1993). This is in agreement with clinical experience which shows that lung complications only occur very rarely in pure hemorrhagic shock (Schlag et al., 1993 see above).

In order to determine the frequency and severity of post-traumatic lung failure it was necessary to observe the animals (named: SELEC 971, SELEC 979 (treated); and Co 968, Co 969, Co 970 (control)) over several days; however, for ethical reasons, it was not possible to induce bone fractures in conscious animals and leave them untreated for several days so that in this subchronic model the tissue trauma is simulated. The activation of the complement system appears to be the earliest trigger for the activation of cellular systems and plays a key role in the rapid occurrence of a non-bacterial inflammatory reaction of the body (Schlag et al., 1993, supra). Therefore, in the model, complement was activated by cobra venom factor.

The mortality for multiple organ failure after severe polytrauma is given as 15 - 30 % in the relevant literature. In the present animal model the severity of the polytrauma was increased to the extent that the mortality is at least twice as high and occurred earlier than in humans. Therefore the observation period was limited to three days.

Adult baboons with a body weight (BW) between 18 and 22 kg were admitted to the study after three months quarantine. The fasted animals were sedated with ketamine (6-8 mg/kg), subsequently intubated and attached to a CPAP respirator (continuous positive airway pressure) (inspiratory O₂ concentration of 25 ± 2%). The anesthesia was maintained with 1-3 mg/kg/h pentobarbital. The animals breath spontaneously. A Swan Ganz catheter was pushed forward into the pulmonary artery via the right femoral vein. A catheter for withdrawing blood and measuring blood pressure was tied into the right arm artery. A large lumen catheter is introduced into the femoral artery for the temporary collection of blood. A catheter for infusions, medication and blood collection was introduced into the left arm vein. The bladder was catheterized for the

measurement of urine production. The Swan Ganz catheter and the arterial catheter were left for three days. For fluid balance the animals received 5 ml/kg/h Ringer solution (electrolyte solution for parenteral liquid substitution) during the anaesthetic phase. The blood temperature of the animals was kept at $\geq 37^{\circ}\text{C}$ with the aid of an infrared lamp. Blood gas analyses were carried out (pO_2 , pCO_2 , pH , BE , HCO_3^-) and hemodynamic parameters were determined (MAP, RAP, PAP, CO, HR). Lung function was determined by means of the respiratory rate (RR) and end expiratory CO_2 . Blood samples were collected repeatedly in order to measure the number of white blood cells (WBCs). Cobra venom factor was administered at a dose of 10 U/kg per i.v. at the beginning of the retransfusion and administered again at a dose of 5 U/kg 1 hour after beginning the retransfusion of the blood. The blood withdrawal for triggering the hypovolemia was regulated such that the MAP (mean arterial pressure) came to lie between 40 and 50 mm Hg and the CO (cardiac output) is reduced by 50 to 70%. Approximately 50 ml/kg blood were usually withdrawn for this and stored until retransfusion. The deficient circulation was maintained for two to three hours and was controlled in such a way that the base excess

was no more than -5 to -7 mEq. At the end of this shock phase retransfusion of the previously collected blood was begun. This phase lasted 4 hours.

The retransfusion was complemented by an additional administration of Ringer solution. Humanized antibody HuDreg 55 or the corresponding volume of saline solution as a placebo was administered intravenously 15 minutes after the start of retransfusion. Anti-L-selectin antibody was administered at a dose of 2 mg/kg. At the end of retransfusion the animals were awakened from anaesthesia and were returned to their cages for observation. At times 24 h, 48 h and 72 h a low level of anesthesia was again induced and the measuring parameters were registered and blood was withdrawn. If the animals had not died before the end of the three day observation period, they were then sacrificed and autopsied. The main terminal points of the study were mortality, survival period and organ damage, for example, to the lung.

In the first experiment, three control animals were treated with placebo solution and two with the HuDreg 55 humanized antibody. Of the three control animals, two died

before the end of the three day observation period at 38 h and 41 h whereas both anti-L-selectin treated animals survived. The lung wet weight, as an expression of organ damage, was almost normal in the antibody treated animals (normal values 7-8 g/kg BW) whereas it had increased considerably in all three placebo-treated control animals (Fig. 1). This is due to infiltration of fluid after the permeability disorder. The cardiovascular parameters CO₂ and MAP (Fig. 2 and 3) are better at 24 hours in the surviving animals than in the control animals. The dying control animals also have a negative arterial base excess (BE) indicating a disturbed acid-base balance (Fig. 4). The leucocytosis (increase in white blood cells) observed in the control animals is absent in the antibody animals (Fig. 5).

EXAMPLE 2 - Use Of Anti-L-Selectin Antibody To Reduce Post-Traumatic Mortality

The experiments reported in Example 1, supra, were continued and expanded to include 28 baboons which were randomly assigned to one of two experimental groups conducted as described in Example 1. The baboons received either 2 mg/kg i.v. of anti-L-Selectin antibody or the

appropriate placebo volume-dose as control 15 minutes after initiation of reperfusion after the ischemia period. The main endpoints for statistical analysis of the study were mortality at the end of the 3-day observation period and survival time. Fisher's exact test was used for mortality analysis and the log-rank-test was used for survival time analysis. One-sided p-values (reduction of mortality or prolongation of survival time by active treatment) are reported. The null hypothesis was rejected only when the probability (p) of the calculated statistic was $p < 0.05$.

Anti-L-selectin antibody reduced ($p < 0.05$) mortality from 10 out of 14 (=71%) baboons in the control group to 3 out of 14 (=21%) baboons in the active treatment group at a level of statistical significance. In addition, survival time in the anti-L-Selectin group was prolonged to 64.4 h, whereas animals in the control group died earlier ($p < 0.05$), on an average at 42.1 h. This difference was statistically significant.

The table summarizes the results:

	Mortality	Survival time (h)
Anti-L-Selectin Antibody	3/14*	64.4±4.7*
Placebo-control	10/14	42.4±5.7

- 5 Mean ± standard error of mean;
 n = 14 per group;
 *, p < 0.05 by Fisher's exact test;
 †, p<0.05 by log-rank-test.
 Observation period was 72 h.

0 These data show that early treatment of baboons
 suffering from ischemia-reperfusion injuries due to
 hemorrhagic-traumatic shock with administration of anti-L-
 Selectin significantly prolongs survival time and reduces
 mortality as compared to placebo-control.

5 EXAMPLE 3 - Use of Anti-L-Selectin Antibody To
Reduce Organ Damage After
Extracorporeal Blood Circulation

0 The protective action of a humanized antibody against
 L-selectin, preferably HuDreg 55, in reducing organ damage
 after extracorporeal blood circulation such as that which
 typically occurs after long operating periods of the
 heart-lung machine in cardiac surgery was studied.

As a model, severe lung damage was caused in baboons by letting the heart-lung machine, which takes over the function of the lungs and heart after the heart is stopped, run for several hours. After the machine was turned off, the pumping action of the heart was resumed, and endogenous circulation and respiration restarted, massive infiltration of activated leukocytes into the pulmonary circulation caused severe damage to the lungs. The leukocytes present in the pulmonary circulation locally release toxic mediators at a high concentration which led to damage of the vascular endothelium with subsequent increase in permeability. In this process fluid crossed over from the vascular space into the alveoli (smallest pulmonary alveoli) which led to an accumulation of fluid in the lung. This impeded gas exchange in the lung and artificial respiration becomes necessary. The oxygen demand increased as the impairment in gas exchange increases in severity and this was further aggravated by a fibroproliferative transformation of the alveolo-endothelial barrier. Thus, in particularly severe cases, the concentration of inhaled oxygen in the respiratory air which is usually about 20 % has to be increased to about 100 %. Nevertheless, in such cases, the supply of pure oxygen is insufficient to

maintain the arterial oxygen concentration or oxygen partial pressure in the blood (paO₂) at an adequate level.

The fibroproliferative transformation process and pulmonary edema result in an increase in the pressures in the arteria pulmonalis which is connected to the lung and this leads to a strain on the right heart. If these reactions build up further this finally leads to death by heart-lung failure.

Adult baboons with a body weight (BW) between 18 and 22 kg are admitted to the study after three months quarantine. The fasted animals were sedated with ketamine (6-8 mg/kg), intubated, and attached to a CPAP respirator (inspiratory O₂ concentration of 25 ± 2%). The anesthesia was maintained with 1-3 mg/kg/h pentobarbital. The animals breathed spontaneously. A Swan Ganz catheter was pushed forward into the pulmonary artery via the right femoral vein. A catheter for withdrawing blood and measuring blood pressure was tied into a right arm artery. A catheter for infusions, medication and blood collection was introduced into a left arm vein. The bladder is catheterized to measure the production of urine. For fluid balance the

animals receive 5 ml/kg/h Ringer solution. The temperature of the animals is maintained at 37°C with the aid of an infrared lamp. Blood gas analyses are carried out (pO_2 , pCO_2 , pH, BE, HCO_3^-) and hemodynamic parameters are determined (MAP, RAP, PAP, CO, HR). The lung function is determined by means of the respiratory rate (RR) and end expiratory CO_2 . Blood samples are collected repeatedly in order to measure the number of white blood cells (WBC).

At the start of the experiment the thorax was opened (thoracotomy) and the vena cava and the aorta was prepared. Afterwards, first the vena cava and then the aorta were cannulated so that blood from the vena cava flowed into the heart-lung machine and later back into the aorta. A peristaltic pump assumes the pumping function of the heart in the heart-lung machine and ensures maintenance of the pressure gradient required for circulation. Exchange of oxygen and binding of carbon dioxide is achieved by membrane oxygenation. The blood was heparinized so that the tubes and blood vessels do not get blocked. The blood flows back to the aorta via the tube system and is distributed in the body via the normal vascular system.

The heart-lung machine takes over the function of the heart and lung. The heart is stopped while the machine is in operation so that the operating surgeon can for example work on the cardiac valves (insert prostheses).

5 Fifteen minutes before the end of the four hour
extra-corporeal circulation, a dose of 2 mg/kg HuDreg 55 or
the same volume dose of placebo was administered directly
into the tube system of the heart-lung machine. The animal
was observed for a further four hours after ending the
extracorporeal circulation. Measurements are carried out
repeatedly before, during and after the extra-corporeal
circulation. In particular arterial blood gases and
parameters for acid-base balance are recorded,
cardiovascular parameters such as the mean arterial blood
pressure, right atrial pressure, pulmonary artery pressure,
cardiac output and heart rate are determined, the lung
function is measured (e.g. end expiratory CO₂) and blood
samples are withdrawn for hematological, clinical-chemical
(e.g. kidney and liver function) and biochemical analyses.
20 In addition, urine production (kidney function) is
measured. Further, parameters for permeability disorders
in the lung were determined. At the end of the experiment,

the animals were sacrificed and necropsy and histological examinations were carried out in order to determine the degree of damage to the various organs and systems such as heart, lung, liver, kidney, intestine, CNS, blood etc. It

5 is expected that the animals treated with HuDreg 55 sustain less organ damage than those treated with placebo.

00047410 020604
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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANTS: MARTIN, Ulrich; HASELBECK, Anton; SCHUMACHER, Guenther;
CO, Man S.
- (ii) TITLE OF INVENTION: ANTI-L-SELECTIN ANTIBODIES FOR PREVENTION OF
MULTIPLE ORGAN FAILURE AFTER POLYTRAUMA AND FOR
PREVENTION OF ACUTE ORGAN DAMAGE AFTER
EXTRACORPOREAL BLOOD CIRCULATION
- (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: Felfe & Lynch
 - (B) STREET: 805 Third Avenue
 - (C) CITY: New York
 - (D) STATE: New York
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 10022
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: 3.5" Computer Disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: ASCII, WordPerfect 5.1
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER: 08/578,953
 - (B) FILING DATE: 27-Dec-95
 - (C) CLASSIFICATION: 530
- (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: EP 95 112 895.8
 - (B) FILING DATE: 17-Aug-95
 - (A) APPLICATION NUMBER: EP 95 114 969.9
 - (B) FILING DATE: 19-Sep-95
- (viii) ATTORNEY/AGENT INFORMATION
- (A) NAME: Hanson, Norman D.
 - (B) REGISTRATION NUMBER: 30,946
 - (C) REFERENCE/DOCKET NUMBER: BOER 1059-PFF/NDH/SLH
- (ix) TELECOMMUNICATION INFORMATION
- (A) TELEPHONE: (212) 688-9200
 - (B) TELEFAX: (212) 838-3884

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 654 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..654

GAC	ATT	CAG	ATG	ACC	CAA	TCT	CCG	AGC	TCT	TTG	TCT	GCG	TCT	GTA	GGG	48
Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	
1				5					10					15		
GAT	AGG	GTC	ACT	ATC	ACC	TGC	AAG	GCC	AGC	CAA	AGT	GTT	GAT	TAT	GAT	96
Asp	Arg	Val	Thr	Ile	Thr	Cys	Lys	Ala	Ser	Gln	Ser	Val	Asp	Tyr	Asp	
			20					25					30			
GGT	GAT	AGT	TAT	ATG	AAC	TGG	TAC	CAA	CAG	AAA	CCA	GGA	AAG	GCA	CCC	144
Gly	Asp	Ser	Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	
		35				40						45				
AAG	CTT	CTC	ATC	TAT	GCT	GCA	TCC	AAC	CTA	GAA	TCT	GGT	ATC	CCA	TCC	192
Lys	Leu	Leu	Ile	Tyr	Ala	Ala	Ser	Asn	Leu	Glu	Ser	Gly	Ile	Pro	Ser	
	50					55					60					
AGG	TTT	AGT	GGC	AGT	GGG	TCT	GGG	ACA	GAC	TTC	ACC	CTC	ACC	ATC	TCT	240
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	
65					70					75					80	
TCT	CTG	CAG	CCG	GAG	GAT	TTC	GCA	ACC	TAT	TAC	TGT	CAG	CAA	AGT	AAT	288
Ser	Leu	Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Asn	
				85					90					95		
GAA	GAT	CCG	TGG	ACG	TTC	GGT	CAA	GGC	ACC	AAG	GTG	GAA	ATC	AAA	CGA	336
Glu	Asp	Pro	Trp	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg	
		100					105					110				
ACT	GTG	GCT	GCA	CCA	TCT	GTC	TTC	ATC	TTC	CCG	CCA	TCT	GAT	GAG	CAG	384
Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	
		115					120					125				
TTG	AAA	TCT	GGA	ACT	GCC	TCT	GTT	GTG	TGC	CTG	CTG	AAT	AAC	TTC	TAT	432
Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	
	130					135					140					


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CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG 480
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145                150                155                160

GGT AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC 528
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
                165                170                175

TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA 576
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
                180                185                190

CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC 624
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
                195                200                205

GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT 654
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210                215

```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 218
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1                5                10                15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
                20                25                30

Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
 35                40                45

Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Ile Pro Ser
 50                55                60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 65                70                75                80

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Asn
                85                90                95

Glu Asp Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100                105                110

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Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
 195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1329 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION:1,,1329

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION:1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GAA GTG CAA CTG GTG GAG TCT GGG GGA GGC TTA GTG CAG CCT GGA GGA 48
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

AGC TTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT ACC TAT 96
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
 20 25 30

GCC ATG TCT TGG GTT CGC CAG GCT CCA GGG AAG GGA CTC GAG TGG GTC 144
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

109917410:03290

GCA	TCC	ATT	AGT	ACT	GGT	GGT	AGC	ACC	TAC	TAT	CCA	GAC	AGT	GTG	AAG	192
Ala	Ser	Ile	Ser	Thr	Gly	Gly	Ser	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys	
	50					55					60					
GGC	CGA	TTC	ACC	ATC	TCC	AGA	GAT	AAT	GCC	AAG	AAC	ACC	CTG	TAC	CTG	240
Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Leu	Tyr	Leu	
	65				70				75						80	
CAA	ATG	AAT	TCT	CTG	AGG	GCT	GAG	GAC	ACG	GCC	GTG	TAT	TAC	TGT	GCA	288
Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	
				85					90					95		
AGA	GAC	TAT	GAC	GGG	TAT	TTT	GAC	TAC	TGG	GGC	CAA	GGC	ACC	CTG	GTC	336
Arg	Asp	Tyr	Asp	Gly	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	
			100				105						110			
ACA	GTC	TCC	TCA	GCT	TCC	ACC	AAG	GGC	CCA	TCC	GTC	TTC	CCC	CTG	GCG	384
Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	
		115					120					125				
CCC	TGC	TCC	AGG	AGC	ACC	TCC	GAG	AGC	ACA	GCC	GCC	CTG	GGC	TGC	CTG	432
Pro	Cys	Ser	Arg	Ser	Thr	Ser	Glu	Ser	Thr	Ala	Ala	Leu	Gly	Cys	Leu	
	130					135					140					
GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA	GGC	480
Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	
	145				150				155						160	
GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	528
Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	
				165					170					175		
GGA	CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	576
Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	
			180				185					190				
GGC	ACG	AAG	ACC	TAC	ACC	TGC	AAC	GTA	GAT	CAC	AAG	CCC	AGC	AAC	ACC	624
Gly	Thr	Lys	Thr	Tyr	Thr	Cys	Asn	Val	Asp	His	Lys	Pro	Ser	Asn	Thr	
		195					200					205				
AAG	GTG	GAC	AAG	AGA	GTT	GAG	TCC	AAA	TAT	GGT	CCC	CCA	TGC	CCA	TCA	672
Lys	Val	Asp	Lys	Arg	Val	Glu	Ser	Lys	Tyr	Gly	Pro	Pro	Cys	Pro	Ser	
	210					215					220					
TGC	CCA	GCA	CCT	GAG	TTC	CTG	GGG	GGA	CCA	TCA	GTC	TTC	CTG	TTC	CCC	720
Cys	Pro	Ala	Pro	Glu	Phe	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	
	225				230				235						240	
CCA	AAA	CCC	AAG	GAC	ACT	CTC	ATG	ATC	TCC	CGG	ACC	CCT	GAG	GTG	ACG	768
Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	
				245					250					255		

TGC GTG GTG GTG GAC GTG AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC 816
 Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
 260 265 270

TGG TAC GTG GAT GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG 864
 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
 275 280 285

GAG GAG CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC 912
 Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
 290 295 300

CTG CAC CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC TCC 960
 Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
 305 310 315 320

AAC AAA GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC AAA GCC AAA 1008
 Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
 325 330 335

GGG CAG CCC CGA GAG CCA CAG GTG TAC ACC CTG CCC CCA TCC CAG GAG 1056
 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
 340 345 350

GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC 1104
 Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 355 360 365

TAC CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG 1152
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 370 375 380

AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC 1200
 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 385 390 395 400

TTC CTC TAC AGC AGG CTA ACC GTG GAC AAG AGC AGG TGG CAG GAG GGG 1248
 Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
 405 410 415

AAT GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC 1296
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 420 425 430

ACA CAG AAG AGC CTC TCC CTG TCT CTG GGT AAA 1329
 Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
 435 440

0094740-07421560

(i) SEQUENCE CHARACTERISTICS:

- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Glu 1	Val	Gln	Leu	Val 5	Glu	Ser	Gly	Gly	Gly 10	Leu	Val	Gln	Pro	Gly 15	Gly
Ser	Leu	Arg	Leu 20	Ser	Cys	Ala	Ala	Ser	Gly 25	Phe	Thr	Phe	Ser 30	Thr	Tyr
Ala	Met	Ser 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Lys	Gly	Leu 45	Glu	Trp	Val
Ala	Ser 50	Ile	Ser	Thr	Gly	Gly 55	Ser	Thr	Tyr	Tyr	Pro 60	Asp	Ser	Val	Lys
Gly 65	Arg	Phe	Thr	Ile	Ser 70	Arg	Asp	Asn	Ala	Lys 75	Asn	Thr	Leu	Tyr	Leu 80
Gln	Met	Asn	Ser	Leu 85	Arg	Ala	Glu	Asp	Thr 90	Ala	Val	Tyr	Tyr	Cys	Ala
Arg	Asp	Tyr	Asp 100	Gly	Tyr	Phe	Asp	Tyr 105	Trp	Gly	Gln	Gly	Thr 110	Leu	Val
Thr	Val	Ser 115	Ser	Ala	Ser	Thr	Lys 120	Gly	Pro	Ser	Val	Phe 125	Pro	Leu	Ala
Pro 130	Cys	Ser	Arg	Ser	Thr 135	Ser	Glu	Ser	Thr	Ala	Ala 140	Leu	Gly	Cys	Leu
Val 145	Lys	Asp	Tyr	Phe	Pro 150	Glu	Pro	Val	Thr	Val 155	Ser	Trp	Asn	Ser	Gly 160
Ala	Leu	Thr	Ser	Gly 165	Val	His	Thr	Phe	Pro 170	Ala	Val	Leu	Gln	Ser 175	Ser
Gly	Leu	Tyr	Ser 180	Leu	Ser	Ser	Val	Val 185	Thr	Val	Pro	Ser	Ser 190	Ser	Leu
Gly	Thr	Lys 195	Thr	Tyr	Thr	Cys	Asn 200	Val	Asp	His	Lys	Pro 205	Ser	Asn	Thr
Lys	Val 210	Asp	Lys	Arg	Val	Glu 215	Ser	Lys	Tyr	Gly	Pro 220	Pro	Cys	Pro	Ser

Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
 225 230 235 240
 Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
 245 250 255
 Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
 260 265 270
 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
 275 280 285
 Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
 290 295 300
 Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
 305 310 315 320
 Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
 325 330 335
 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
 340 345 350
 Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 355 360 365
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 370 375 380
 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 385 390 395 400
 Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
 405 410 415
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 420 425 430
 Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
 435 440

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 220 amino acid residues
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Thr Ile Thr Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
 20 25 30
 Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
 35 40 45
 Ala Pro Lys Leu Leu Val Tyr Phe Ala Ser Thr Arg Glu Ser Gly Val
 50 55 60
 Pro Asp Arg Phe Ile Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 65 70 75 80
 Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Phe Cys His Gln
 85 90 95
 His Tyr Ser Thr Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Val
 100 105 110
 Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
 115 120 125
 Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
 130 135 140
 Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
 145 150 155 160
 Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
 165 170 175
 Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
 180 185 190
 Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
 195 200 205
 Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215 220

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 448 amino acid residues
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1 5 10 15
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30
 Val Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45
 Gly Tyr Ile Tyr Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
 50 55 60
 Lys Gly Arg Val Thr Ile Thr Ser Asp Glu Ser Thr Asn Thr Ala Tyr
 65 70 75 80
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Glu Glu Tyr Gly Asn Tyr Val Arg Tyr Phe Asp Val Trp Gly
 100 105 110
 Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
 115 120 125
 Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
 130 135 140
 Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
 145 150 155 160
 Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
 165 170 175
 Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
 180 185 190
 Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His
 195 200 205
 Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly
 210 215 220
 Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser
 225 230 235 240
 Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
 245 250 255
 Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro
 260 265 270
 Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
 275 280 285

T09220-0742650

Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val
 290 295 300
 Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
 305 310 315 320
 Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr
 325 330 335
 Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
 340 345 350
 Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
 355 360 365
 Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
 370 375 380
 Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
 385 390 395 400
 Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser
 405 410 415
 Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
 420 425 430
 Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
 435 440 445

000140-073604
 109220-074200